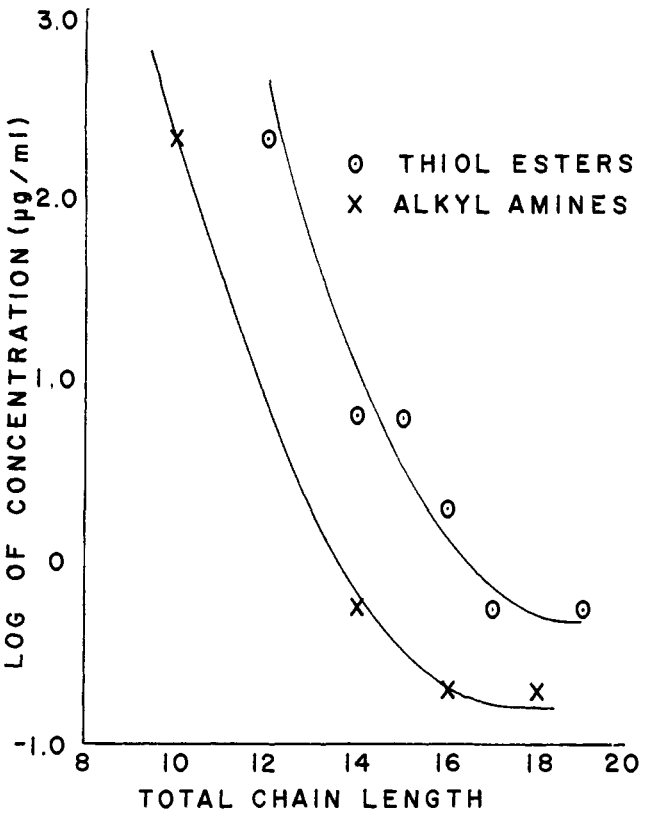

Figure 2.—A comparison of the concentration of β-alanyl thioesters and long chain alkylamines required to completely inhibit the growth of *Lactobacillus arabinosus*. The legend indicates the total number of atoms in each of the chains attached to the amino group.

cantly less toxic. These data suggest that the inhibitory properties of the thio esters are a function of their lipid character rather than of their alkylating ability.

Experimental Section[10]

Biological Testing Procedures.—The organisms used in this study were *E. coli* 9723, *L. arabinosus* 17-5, *L. casei* 7469, and *P. cerevisiae* 8042. An inorganic salts medium was used for *E. coli*,[11] and a previously reported amino acid medium was utilized for *L. arabinosus* and *L. casei*[12] except that the vitamin supplement contained 0.2 μg/ml of calcium pantothenate, and 500 μg of glutamic acid was added per tube for assays with *L. casei*. An acid-hydrolyzed casein medium[13] was used for *P. cerevisiae* with the Salts A concentration increased fourfold. Growth assay times and temperatures were 16 hr at 37° for *E. coli*, 24 hr at 37° for *L. casei*, 20 hr at 30° for *L. arabinosus*, and 21 hr at 30° for *P. cerevisiae*. The amount of growth was determined on a Bausch and Lomb Model 20 spectrometer as per cent transmission at 600 mμ.[14]

β-Alanyl Thio Ester Hydrochlorides.—A 20-ml sample of the appropriate thiol was cooled to appoximately 5°, and 3 g of β-alanyl chloride·HCl[8] was added. The reaction mixture, protected from moisture, was stirred for 15 min at ice bath temperature and then allowed to come to room temperature. Stirring was continued overnight. The mixture was again cooled to 5°, and Et_2O (50 ml) was added to give the desired product which was filtered and washed with dry Et_2O. The resulting solids were recrystallized (MeOH–Et_2O) to yield hygroscopic compounds which were maintained in a desiccator. (See Table I).

***n*-Decyl 3-Aminopropionate Hydrochloride.**—The same procedure was used for this compound as for the thio ester. The solid had mp 84–85° and analyzed satisfactorily for C, H, and N.

(10) Melting points were determined with a Thomas–Hoover Capillary Melting Point Apparatus. The authors are indebted to Mrs. Sarah R. Bryant for assistance with the microbial testing procedures.

(11) E. H. Anderson, *Proc. Nat. Acad. Sci., U.S.*, **32**, 120 (1945).

(12) J. M. Ravel, L. Woods, B. Felsing, and W. Shive, *J. Biol. Chem.*, **206**, 391 (1954).

(13) E. M. Lansford, Jr., W. M. Harding, and W. Shive, *Arch. Biochem. Biophys.*, **73**, 180 (1958).

(14) R. E. Masingale, S. R. Bryant, C. G. Skinner, J. Nash, and P. F. Kruse, Jr., *J. Med. Chem.*, **12**, 152 (1969).

Metal Complexes of 1-Substituted 3-Hydroxyureas

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A considerable amount of work has been done to indicate the existence of a definite correlation between the metal binding properties of therapeutically active compounds and their activity.[2–4] For instance, therapeutically active analogs of tetracycline form 2:1 complexes with Cu (II), Ni (II), and Zn(II) ions whereas the inactive analogs appear to form only 1:1 complexes.[5] Hydroxyurea (HU)[6] and ethylhydroxyurea (EHU)[7] have been found to be very active against L1210 lymphoid leukemia. When patients with myelogenous leukemia were placed on HU therapy, acetohydroxamic acid was identified in their blood. To explain the mode of action of HU in biological systems, two theories have been proposed. According to Fishbein and Carbone,[8] HU is hydrolyzed to hydroxylamine, which cleaves thio esters such as acetyl-coenzyme A, as shown in eq 1. A second theory[9] suggested that

$$\begin{aligned} &NH_2CONHOH + 2H_2O \longrightarrow NH_2OH + NH_4^+ + HCO_3^- \\ &NH_2OH + CH_3COSCoA \longrightarrow CH_3CONHOH + HS\text{-}CoA \end{aligned} \quad (1)$$

hydroxyurea caused fragmentation of isolated DNA and induced chromosomal abnormalities in mammalian cells. The proposed mechanism is shown in eq 2.

$$NH_2CONHOH \xrightarrow[-H_2O]{\text{oxidation}} 2(HON:) \longrightarrow H_2N_2O_2 \quad (2)$$

Hyponitrous acid ($H_2N_2O_2$) like hydroxylamine and other compounds, causes cleavage of the main chain of cellular DNA. Our results based on the decomposition of Fe(III)–EHU complex suggest that hydroxyureas may be involved in an oxidation–reduction reaction, producing NO. In this paper, we also wish to describe the synthesis, metal-binding properties, and antitumor activity of several 1-substituted 3-hydroxyureas.

The synthesis of 1-substituted 3-hydroxyureas **1a–g** was achieved by a slight modification of the method

(1) J. C. Dabrowiak, M. A. Thesis, Western Michigan University, Kalamazoo, Mich., 1967.

(2) J. T. Doluisio and A. N. Martin, *J. Med. Chem.*, **6**, 20 (1963).

(3) R. C. Warner and I. Weber, *J. Amer. Chem. Soc.*, **75**, 5094 (1953).

(4) K. W. Kohn, *Nature*, **191**, 1156 (1961).

(5) J. T. Doluisio and A. N. Martin, *J. Med. Pharm. Chem.*, **6**, 16 (1963).

(6) B. Stearns, K. Losee, and J. Bernstein, *ibid.*, **6**, (2), 201 (1963).

(7) Private communication, Cancer Chemotherapy National Service Center, Bethesda, Md.

(8) W. Fishbein and P. Carbone, *Science*, **142**, 1069 (1963).

(9) A. Bendich, E. Borenfreund, G. Korngold, and M. Krim, *J. Nat. Cancer Inst.*, **32**, 667 (1964).

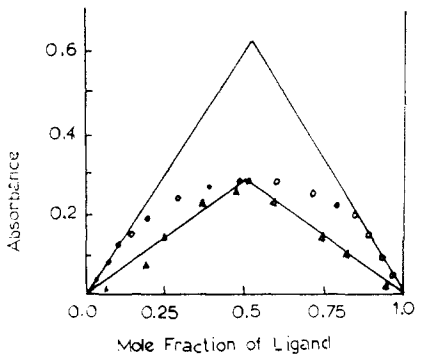

Figure 1.—Continuous variation plots: (○) iron(III)-EHU complex in EtOH; (△) iron(III)-HU complex in H_2O.

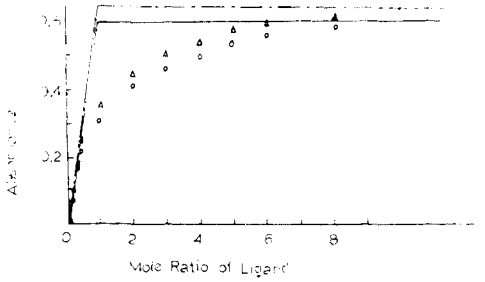

Figure 2.—Molar ratio plots: (○) iron(III)-EHU complex in EtOH; (△) iron(III)-HU complex in EtOH.

reported by Dresler and Stein.[10] Treatment of an aqueous solution of hydroxylamine with the appropriate isocyanate (eq 3) afforded the corresponding hydroxyureas, **1a–g**. The ability of these hydroxyureas to form complexes with various metal ions was studied by mixing equimolar amounts of compounds **1a–g** with

$$NH_2OHHCl + RN{=}CO \xrightarrow{NaOH} \underset{\textbf{1a–g}}{RNHCONHOH} \quad (3)$$

1a, R = $CH_3OC_6H_4$-*o*
b, R = $CH_3C_6H_4$-*o*
c, R = ClC_6H_4-*m*
d, R = *n*-Pr
e, R = 
f, R = H
g, R = Et

suitable metal salts in absolute EtOH or H_2O. However, of the seven hydroxyureas studied, only two, HU (**1f**) and EHU (**1g**) appeared to form complexes with Fe(III) and Cu (II) ions. Only the Cu(II)–HU complex was isolated in crystalline form. The Fe(III)–HU and Fe(III)–EHU complexes were not stable enough to be isolated. Therefore, their formation was detected by spectrophotometric methods. In these complexes, the metal to ligand ratio was determined using the continuous variation method.[11] As shown in Figure 1, by plotting the absorbance of the solution *vs.* the mole fraction of the ligand for Fe (III)–HU and Fe (III)–EHU complexes, two maxima were obtained. The metal to ligand ratio was obtained by extrapolating the extreme linear portions until they crossed. Thus in each case, the metal to ligand ratio was found to be 1:1. Furthermore, the molar ratio method of Yoe and Jones[12] was used to determine the stability constants of Fe(III)–HU and Fe(III)–EHU complexes. Figure 2 shows the plots of absorbance *vs.* molar ratio of ligand for these complexes. The stability constants for Fe (III)–HU and Fe(III)–EHU complexes were found to be log K_{st} 3.65 and 3.47, respectively. The decomposition of Fe(III)–EHU complex in H_2O led to the evolution of a gas which was identified by ir spectroscopy to be NO. Therefore, it appears that the hydroxyureas are involved in an oxidation–reduction process, producing NO.

The seven 1-substituted 3-hydroxyureas **1a–g** were submitted to Cancer Chemotherapy National Service Center (CCNSC) for testing. The analytical as well as antitumor activity data are summarized in Table I. Of the seven hydroxyureas studied, only HU and EHU were associated with biological activity. Strangely enough, these were the only two hydroxyureas which showed any metal-binding properties. Obviously, the present data are insufficient to draw any structure–activity correlations or to correlate metal complexing ability to biological activity of 1-substituted 3-hydroxyureas.

Experimental Section

Melting point were determined with a Thomas-Hoover Uni-Melt apparatus and are corrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values. Ir spectra were obtained on a Beckman IR-8 recording spectrophotometer using Nujol mulls, uv spectra on a Cary-14 recording spectrophotometer.

1-Substituted 3-Hydroxyureas, General Procedure.—All the 1-substituted 3-hydroxyureas (**1a–g**) were prepared by the method of Dresler and Stein.[10] In a typical experiment, 40 ml of a solution of 13.9 g (0.20 mol) of $NH_2OH \cdot HCl$ was added to 25 ml of 0.2 *M* NaOH. The resulting solution was placed in an ice bath and stirred vigorously while 0.2 mol of the appropriate isocyanate was added to it dropwise over a period of 15–20 min. Stirring was continued for 45 min, and the white precipitate thus formed was filtered and recrystallized from $CHCl_3$ or $(ClCH_2)_2$. The yields, melting points, and analyses are given in Table I.

Preparation of Complexes. Cu(II)–HU Complex.—An ethanolic solution (25 ml) containing 2.97 g of $Cu(ClO_4)_2 \cdot 6H_2O$ was added to a solution of 0.62 g of HU in 25 ml of abs EtOH. The resulting olive green ppt was filtered to yield 1 g of bis(hydroxyurea)copper(II) perchlorate, $Cu(HU)_2(ClO_4)_2$. *Anal.* ($C_2H_8Cl_2CuN_4O_{12}$) C, 5.81; H, 1.94; N, 13.63. Found: C, 6.11; H, 2.14; N, 14.19.

Fe(III)–EHU Complex.—A solution (25 ml) containing 2.018 g of $FeCl_3 \cdot 6H_2O$ in EtOH was mixed with an equal volume of a 3.3×10^{-1} *M* solution of EHU in EtOH. The solvent was quickly evaporated off at reduced pressure. The resulting oil was dissolved in EtOAc to afford a white residue which was removed by filtration. Filtration and removal of the solvent at reduced pressure yielded a dark green oil which failed to crystallize out. However, the formation of Fe(III)–EHU complex was detected by spectrophotometric methods as discussed later.

Decomposition of Fe(III)–EHU Complex in H_2O.—An aq solution (15 ml) containing 0.7662 g of EHU was added to an

(10) W. Dresler and R. Stein, *Justus Liebigs Ann. Chem.*, **150**, 242 (1969).
(11) H. Willard, L. Merrit, and J. Dean, "Instrumental Methods of Analysis," D. Van Nostrand Co., Princeton, N. J., 3rd ed, 1958, p 568.
(12) J. Yoe and A. Jones, *Ind. Eng. Chem. Anal. Ed.*, **16**, 111 (1961).

TABLE I

ANTITUMOR ACTIVITY[a] OF 1-SUBSTITUTED 3-HYDROXYUREAS (**1a–g**)

Compd	Mp (°C)	Yield (%)	Formula	Analysis	Test[b] system	Dose (mg/kg)	Survivors	Tumor weight[c] —Survival (days)— Test	Control	Per cent (T/C)
1a	171–173	29[d]	$C_8H_{10}N_2O_3$	C, H, N	LE	400	4/4	9.5	9.5	100
					WM	400	6/6	4.5	5.7	78
1b	146–147	43[e]	$C_8H_{10}N_2O_2$	C, H, N	LE	400	4/4	10.0	9.5	105
					LE	200	4/4	9.5	9.5	100
1c	134–136	46[e]	$C_7H_7ClN_2O_2$	C, H, N	LE	400	4/4	9.5	9.5	100
					LE	200	6/6	9.7	9.4	103
					WM	400	4/6	1.0	5.7	17
1d	128–129	31[e]	$C_4H_{10}N_2O_2$	C, H, N	LE	400	4/6	12.5	9.6	130
						200	6/6	10.8	8.7	124
					WM	400	6/6	6.8	8.9	76
1e	116–118	16[d]	$C_7H_{14}N_2O_2$	C, H, N	LE	400	6/6	9.2	9.6	95
					WM	400	6/6	5.0	5.7	87
1f	140–142	28[d]	$CH_4N_2O_2$	C, H, N	LE[f]	200		12.3	8.1	151
						400		16.2	8.1	200
1g	125–126	43[e]	$C_3H_8N_2O_2$	C, H, N	LE[f]	200	6/6	12.0	9.4	127
						400	6/6	12.5	9.1	137
					SA[f]	375	6/6	808	1185	68
					CA[f]	337	10/10	680	1536	44

[a] Testing was done at Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md. See *Cancer Chemother. Rep.*, **25**, 1, 10 (1962). [b] LE, L-1210 lymphoid leukemia; WM, Walker carcinosarcoma 256; SA, sarcoma 180; CA, adenocarcinoma 755. [c] For test systems SA and CA total tumor weight in grams: for test systems LE and WM, survival time in days. [d,e] Recrystallization solvents chloroform and 1,2-dichloroethane, respectively. [f] See ref 6.

equal vol of $FeCl_3 \cdot 6H_2O$ (2.0557 g) in H_2O. The gas that evolved from the blue solution was bubbled through 10 ml of cold (0–5°) Et_2O using a gas dispersion tube. Five minutes after mixing, the reaction vessel was heated to 50° for 10 min, producing a nearly colorless solution. An ir spectrum of the Et_2O solution showed strong absorptions at 2335, 665 (CO_2), 2280 (N=C=O), and 2220 (N≡N→O) cm^{-1}.

Fe(III)–HU Complex.—A 7.06×10^{-1} *M* solution (25 ml) of $FeCl_3 \cdot 6H_2O$ in abs EtOH was added to an equal vol of an EtOH solution of 7.06×10^{-1} *M* HU. The solvent from the blue solution was then evaporated off at reduced pressure and the resulting dark blue oil dissolved in 2 ml of abs EtOH. The colorless residue was filtered off and the filtrate on removal of the solvent at reduced pressure gave a dark green oil. As before, the formation of Fe(III)–HU complex was shown by spectrophotometric methods.

Spectrophotometric Determination of Fe(III)–HU and Fe(III)–EHU Complexes. Continuous Variation Method. Fe(III)–HU Complex in H_2O.—Aqueous solutions (250 ml) of 2×10^{-2} *M* $FeCl_3 \cdot 6H_2O$ and HU were prepared and appropriate volumes of each were added to ten 25-ml volumetric flasks to give the following mole fractions of ligand: 0.8, 0.20, 0.28, 0.40, 0.48, 0.52, 0.60, 0.72, 0.80, and 0.92. The solutions were mixed immediately prior to the determination of the absorbance at 560 mμ. The results are plotted in Figure 1 as absorbance *vs.* mole fraction of the ligand.

Fe(III)–EHU Complex in EtOH.—Appropriate volumes of 1×10^{-3} *M* abs EtOH solutions of EHU and $FeCl_3 \cdot 6H_2O$ were added to sixteeen 25-ml volumetric flasks such that the following mole fractions of ligand, 0.40 0.08, 0.12, 0.16, 0.20, 0.40, 0.48, 0.52, 0.60, 0.80, 0.88, and 0.92 resulted. Each solution was mixed immediately prior to the determination of absorbance at 610 mμ. As before, the results are plotted as absorbance *vs.* mole fraction ligand.

Molar Ratio Method.—The molar ratio method of Yoe and Jones[11] was applied to the Fe(III)–HU and Fe(III)–EHU complexes. The results, as shown in Figure 2, showed the formation of 1:1 complex in each case.

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Potential Antineoplastics. III. A Series of 1-Thiocarbamoyl-3-methyl-4-arylazo-5-methyl(or phenyl)pyrazoles

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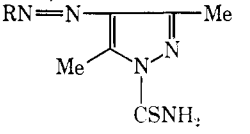
The discovery that isoquinoline-1-carboxaldehyde thiosemicarbazone and its several congeners which possess the N–N–S or O–N–S tridentate ligand system, exhibit substantial antineoplastic activity,[1,2] and the

TABLE I

1-THIOCARBAMOYL-3,5-DIMETHYL-4-ARYLAZOPYRAZOLES



No.	R	Yield %	Mp, °C	Color[a]	Formula	Analyses
1	Ph	88	121–122	OYN	$C_{12}H_{13}N_5S$	N, S
2	2-MePh	85	138–139	YN	$C_{13}H_{15}N_5S$	N, S
3	4-MePh	82	142–143	YN	$C_{13}H_{15}N_5S$	N, S
4	3-ClPh	84	118–119	YN	$C_{12}H_{12}ClN_5S$	N, S, Cl
5	4-ClPh	81	150–151	ON	$C_{12}H_{12}ClN_5S$	N, S, Cl
6	2-EtOPh	78	146–147	YN	$C_{14}H_{17}N_5OS$	N, S
7	4-EtOPh	75	132–133	YP	$C_{14}H_{17}N_5OS$	N, S
8	2-MeOPh	79	147–148	YF	$C_{13}H_{15}N_5OS$	N, S
9	2-NO_2Ph	75	153–154	OYP	$C_{12}H_{12}N_6O_2S$	N, S
10	2,5-Me_2Ph	75	192–193	ON	$C_{14}H_{17}N_5S$	N, S
11	2-Cl-6-MePh	65	167–168	ON	$C_{13}H_{14}ClN_5S$	N, S, Cl

[a] B = bright; F = fibers; G = golden; N = needles; O = orange; P = plate; Pe = pale; Y = yellow.

(1) R. W. Brockman, J. R. Thomson, M. J. Bell, and H. E. Skipper, *Cancer Res.*, **16**, 167 (1956).

(2) F. A. French, and E. J. Blanz, Jr., *ibid.*, **25**, 1454 (1965); **26**, 1638 (1966).